MICROSCOPE OBJECTIVES.

T. J. BURRILL, Ph. D., Champaign, Illinois.

I had the honor of presenting to this Society at its last meeting a paper embodying my experience and opinions concerning the microscope stand. I now wish to offer the results of personal experience in the use of various objectives for microscopical work, especially along the lines followed as a teacher and investigator of biological science.

The task thus set before me is more difficult than that of last year. Little niceties of difference count much more in an objective than in the construction of a stage, or rack and pinion adjustment; and, though one may be sure that his preference is not founded upon fancy, yet may find it hard to state in words upon just what special characteristics he does base his choice. In the paper last year the names of makers were carefully excluded; this time it is impossible to get along without reference by name to the manufacturers of the instruments cited. I heartily wish it could be avoided, and accomplish the purpose intended, for it is a source of embarrassment to myself and is also liable to be seriously misinterpreted. All that can be said in justification of what follows is that I am under obligations to no one, either directly or by implication, except as necessitated by truth and fair dealing, and that all matters of personal interest are thoroughly placed aside, if I am capable of so doing. The articles used are all owned by myself or by the institution in whose service I am, with one somewhat conspicuous exception, and that was loaned me, upon request, for the purpose of this paper. No comparison is made with such as I have not had an abundant opportunity to test, and, with the exception just mentioned, with none that have not been in use during some years of time.

In the paper upon stands, a note was made upon the fact that we are prone to like best that with which we become accustomed. In the case of objectives, however, there is less room for such preference,

because the mere handling of one is practically that of others, including the position and movements of one's body when at work. To be sure, in order to get the very best results with a high quality objective, one must patiently learn how to use that particular instrument, but this is another thing. The force of habit has little to do in this last case, while it is exceedingly strong in the method of moving the object under the lens, and in the manipulations generally of the stand.

It should also be stated that my work has chiefly been upon uncolored objects mounted in water, with or without the addition of carbolic acid or glycerine, and upon colored objects in balsam. The main exception is that of diatoms in balsam, and in this case as a test for the objective rather than work upon the objects for their own sake.

Magnification.

Whatever may be the facts in regard to the use of high-power eyepieces to secure the requisite magnification in mere tests, for long-continued work over the tube anything in the upper end of less than about one inch focal length is unsatisfactory to me. The strain upon the eye is certainly less with the medium and low power oculars, and the image is better to my eye, even with the finest objectives made. I choose, therefore, such focal length in the objective as will give sufficient magnification with a Huyghenian eye-piece amplifying about ten times, as the upper limit. Higher magnification by the eye-piece may be useful in testing an objective, and may, it is true, to some persons be available for long-continued work; but I am making a report of personal experience. The only other thing necessary to say here is that usually the less amplification the better, after a suitable amount is obtained. Hence neither objective nor eye-piece should be of less focal length than will conveniently serve the purpose required. For botanical laboratory use, a one-half inch and a one-fifth inch dry objective is the best selection for the common work of students. Occasionally higher powers are needed, sometimes running up to the highest and best procurable. For these exceptional cases provision should be made by having a few such objectives at hand, but students need not be furnished with them to keep in their desks, as with those first named.

Really serviceable magnification seems to reach its limit in about a one-fifteenth inch, or, at most, an one-eighteenth inch objective. Only in rare cases is anything of higher power than a one-tenth inch

of best quality effectively superseded—with me in nothing but certain studies upon bacteria.

Angle of Aperture.

It appears to me that something similar can be said of the angle of aperture. In the matter of difficult resolution with oblique light, high class and even medium grade objectives have been, in my hands, proportionally successful in just about the order of their aperture, though exceptions have been noted; but for most other uses it does not appear that the angle of aperture should be relatively rated so high in the qualities of objectives. It must not be inferred from this that wide angles are, in and of themselves, injurious for biological work. Other things being equal, I should always prefer them, cheerfully putting up with any lack of penetration, and to a certain extent with inconvenient working distance for the other advantages offered; but crispness of outline, of even the smallest bacteria, depends upon other things quite as much as upon the aperture and cost-price of an objective. These smallest bacteria measure about ½ μ ($\frac{1}{50000}$ inch), or about the distance apart of the dots from center to center of Pleurosigma angulatum. We all know that great angle is not necessary upon objects of this size. The question is whether excess of angle above a certain essential degree is of any importance whatever, or indeed whether an objective of wide aperture is on this account especially superior when the illumination is a narrow beam of axial light. When the object is too small or too slender to be seen by an objective of narrower angle, no doubt can exist even in this last case of the essential advantage of the greater aperture; but unless one wishes to see the flagellum of a Bacillus or the minute structure of a diatom valve, his laboratory work may perhaps be just as successful with first-class objectives of less than the widest angle procurable. Should those of moderate angle possess better definition (not resolution), then for their proper work they are the better lenses. My later purchases for students' ordinary use have been of 110° air angle for a one-fifth inch, with the expectation that anything up to the widest numerical aperture may sometimes be accessible. For the closest possible studies upon the exact size (measurement and shape of small stained bacteria) a Tolles one-fifteenth inch homogeneous immersion of 123° balsam angle is the best I have used, though others at hand have considerably wider aperture.

Get the Best.

Having decided what is most suitable for the work proposed, the very best should be selected for students' use, as well as for special investigations. It may be said that the expense would often be too great, and that cheap instruments or none constitute the alternatives. Often, however, this is a mere outgrowth of too cheap ideas, either on the part of instructors or boards of trustees.

If the real needs are fairly appreciated, in this as in any other case, they can usually be met in some way ; otherwise, how are microscopes obtained at all? At any rate, instructors should inform themselves with the utmost care, and then equip their pupils in the best possible manner with this the most delicate of all tools. No questions of home or foreign manufacture, of accidents of popular approval or of hereditary service, should be allowed weight in the selection of a microscope objective; neither should the cost price be taken as an index of quality. No one can be blamed for buying what he finds to be the best goods for the least money.

Governed by these principles, I have ceased ordering from abroad for students' use. Without naming other makers, I choose the objectives of the Bausch and Lomb Optical Company in preference to those of Leitz. I have in daily use some first-class wide-angled dry objectives of the Gundlach Optical Company that have given most excellent satisfaction. Anxious to have the best, as improvements were announced, I have ordered from time to time five first-class wide-angled objectives, each one supposed at the time to be the very best in the market. This paper may seem less presumptuous with this statement inserted.

Specific Tests.

I am now to report the results of some comparative tests made with certain named objectives under described methods of procedure. When the title of the paper was announced I hoped to have photographs taken in different ways for each objective tried, but have found too much time consumed in other directions to permit it this season. Please permit me to express the conviction that these proposed photographs would have certainly corroborated the statements herein made.

In order to decide with certain correctness of the relative quality of the objectives compared, the tests were purposely made as difficult as circumstances permitted, but under these difficulties each was given

the best handling possible for the manipulation. The objectives, all homogeneous immersion, and that of Zeiss an apochromatic, were as follows:

Tolles' 1-15th (1880) balsam angle, 1.23°; Zeiss' apochromatic 1-12th (1887) N. A., 1.40; Herbert Spencer's 1-10th (1888) balsam angle, 1.30°; Gundlach's 1-12th (1890) balsam angle, 1.36°. The last was asked for and loaned to me for trial. An attempt was also made to include a Leitz's 1-12th (1888) N. A., 1.25°, but it was not possible to use it on the same stand; hence not certainly under same conditions, and so it is not included in this report. I have not been able under fairly similar conditions to make it do what is reported for the others.

The tests were made upon Möller's balsam-mounted test plate, with an ordinary small coal-oil lamp with flat wick three-fifths inch wide and common round chimney, on which, however, was placed a tin extension 16 inches long to improve the combustion and steady the flame. Any one who sits down to a prolonged task of this kind will at least appreciate the latter improvement thus obtained. The lamp was placed 30 inches from mirror to the left, with center of flame, used edgewise, and mirror of the same height. The mirror bar was placed at the angle of 50° from axis of the instrument, and the concave mirror was accurately focused by means of the paper label on the test slide. No sub-stage helps of any kind were used. After adjustment of object, objective, and light, as described, an ordinary bull's-eye, same height of flame and mirror, was pushed in and out at will near the lamp, flat surface to the latter. The tube of the instrument could be closed to 6.5 inches, measured from its lower end to top of draw-tube, and could be elongated to 13 inches. The Tolles and Spencer objectives have screw collars. These were adjusted for their best effect with tube length of 10 inches, measured from front of objective; for the others the tube was varied to suit. It should be said that the Zeiss objective was ordered for the long tube.

Amphipleura pellucida on this particular slide is of medium grade as to difficulty of resolution, but as difficult as any I have seen in Möller's test slides. Number 19 is probably proportionally easier, often showing by light and adjustments which 18 defies. I think this last is unusually difficult, and the same may be said of No. 12, *Grammatophora subtilissima*. The others seem to be fair average shells. As immersion media, somewhat thickened cedar oil, furnished by Zeiss, and a fluid sent out by Gundlach Optical Company, were used

successively with all the objectives, with, however, no perceptible difference in result. The work was done in the daytime, with uncurtained windows behind operator. There were no windows in front or at the sides.

Under these conditions all four objectives resolved Amphipleura so plain that any tyro could make out the transverse lines, at least when the bulls-eye aided the illumination. Often the lines appeared the moment the focus was secured, and this could be changed back and forth with almost certainty that they would be evident whenever the proper adjustment was made. I need not say, however, that it always required careful work and that there were failures as well as triumphs. The two non-adjustable objectives did best with the shortest tube and negative ocular. With Zeiss' compensating ocular the result was rather more satisfactory with the ten-inch tube length. There did not, however, appear to be the same difference with the Gundlach in this respect, the Zeiss eye-piece also showing well with short tube. With the apochromatic at its best the diatom appeared perfectly flat, with midrib and margins showing distinct and clear when the lines were in focus, a thing not to be said of the others, though the Spencer came nearest to it when adjusted at 8, with ten-inch tube. The whole field, too, of the first named, including the object, was beautifully white. With the Gundlach it seemed to me that the lines were as distinct and crisp as with the Zeiss, and could be counted with reliability, a few at a time. When these were best shown the raphe and margins glowed with red, shading to dark, and a little movement of the focus downward was necessary to render the margins most distinct. With a longer tube the lines more evidently stood above the outline. With the Spencer at its best, I found little changes of illumination, etc., destroyed the resolution to a more marked degree than with the two others just named, and, though the lines were beautifully shown and the outline fair at same time, it seemed to me that counting would be a much more difficult undertaking. It should be remembered, however, that the magnification was less, and this I could not fairly make up with higher eye-piecing. Under a solid ¼th-inch ocular I was unable to make any distinction in the quality of the lines. With all the objectives, they were like parallel ropes, with uneven and woolly outlines.

The Tolles objective gave the lines readily enough, but partaking somewhat of the character just described with the other objectives and the solid ocular. With the magnification reduced to that of the 1-12th by the use of longer eye-pieces, the haziness of lines partially

disappeared, but in no way did they seem so beautifully sharp as in the other cases. With both the Spencer and the Tolles there was a tinge of red in the raphe, in some cases merging into a dark shade, when the lines showed best under the manipulation of the screw collar.

Upon the whole, it seemed to me the apochromatic in this special test was really in the lead, though the distinction had to be carefully drawn. The results on other diatoms on the plate were similar, so far as could be determined, the rating of the objectives remaining the same.

I next tried the mirror in exactly central position, with other things remaining the same, save as the height and position of the lamp and bull's-eye required changing. This was varied, too, during same test by inserting a narrow-angled 1-5th-inch dry objective as a condenser, taking great care that it was in central position. In each case (to be further assured that the illumination was axial) examination was made by removing ocular and looking at the bright spot in the back lens of the objective. The difference in the performance of the objectives was certainly less marked than with oblique light. The required tube lengths remained about as before stated, with, however, less noticeable difference in a given amount of change. I obtained a kind of a glimmer of resolution on No. 19 with the apochromatic and Gundlach's lenses, but nothing with any of them on 18 or 20.

The others were well resolved by all four objectives, *Grammatophora subtilissima* giving the most trouble. I have never seen a balsam-mounted Amphipleura resolved by truly central illumination, though others have reported it with several objectives. When using the condenser named, by moving it only a little to one side, lines could be made out, but no comparative tests of this kind were made.

I had previously tried, with the help of an expert assistant, the three objectives in my possession in photographing violet-stained bacteria with central light, showing scarcely appreciable differences, but favoring the Zeiss and Spencer over the Tolles, unless the increased difficulties with the higher power proved too much for the skill of the manipulators.

A few of these results are herewith submitted for your inspection. All were balsam-mounted objects, and all were violet stains. Eastman's orthoscopic plates were used with a tuemeric screen. Some of these objects were among the hardest of the kind to catch by the camera, as a comparison with others will show.

I have now to add a word in regard to the durability of the apo-

chromatic, the want of which has been frequently questioned After about two years' use it became evident that this lens was in some way impaired, and by looking through it from the back with a magnifier a hazy-granular appearance was noticeable, not due to dust on the back lens. Last March the objective was sent to the makers for examination and repair. It reached me again in July as good as new, with the statement that the front lens had been slightly decentered, and that the repair had been easily made, and was without charge. I have no other information upon this point, neither do I know what interpretation to place upon the granular appearance noted. There is certainly nothing of the kind visible now.